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PATENT COOPERATION TREATY

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INTERNATIONAL PRELIMINARY EXAMINATION REPORT

(PCT Article 36 and Rule 70)

Applicant's or agent's file reference PF-0673 PCT	FOR FURTHER ACTION	See Notification of Transmittal of International Preliminary Examination Report (Form PCT/IPEA/416)
International application No. PCT/US00/05153	International filing date *(day/month/year)* 29 FEBRUARY 2000	Priority date *(day/month/year)* 01 MARCH 1999
International Patent Classification (IPC) or national classification and IPC IPC(7): C07H 17/00; C12P 21/06; C07K 14/00 and US Cl.: 536 23.1; 435/6, 69.1; 530/350; 514/2		
Applicant INCYTE PHARMACEUTICALS, INC.		

1. This international preliminary examination report has been prepared by this International Preliminary Examining Authority and is transmitted to the applicant according to Article 36.

2. This REPORT consists of a total of 5 sheets.

   ☐ This report is also accompanied by ANNEXES, i.e., sheets of the description, claims and/or drawings which have been amended and are the basis for this report and/or sheets containing rectifications made before this Authority. (see Rule 70.16 and Section 607 of the Administrative Instructions under the PCT).

   These annexes consist of a total of 0 sheets.

3. This report contains indications relating to the following items:

   I ☒ Basis of the report

   II ☐ Priority

   III ☒ Non-establishment of report with regard to novelty, inventive step or industrial applicability

   IV ☐ Lack of unity of invention

   V ☒ Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

   VI ☐ Certain documents cited

   VII ☐ Certain defects in the international application

   VIII ☐ Certain observations on the international application

Date of submission of the demand 26 SEPTEMBER 2000	Date of completion of this report 25 JANUARY 2001
Name and mailing address of the IPEA/US Commissioner of Patents and Trademarks Box PCT Washington, D.C. 20231 Facsimile No. (703) 305-3230	Authorized officer Helen Cochrane Carlson for HELEN COCHRANE CARLSON, PH.D. Telephone No. (703) 308-0196

Form PCT/IPEA/409 (cover sheet) (July 1998)★

INTERNATIONAL [redacted]LIMINARY EXAMINATION REPORT

International application No.
[redacted]PCT/US00/05153

I. Basis of the report

1. With regard to the **elements** of the international application:*

[X] the international application as originally filed

[X] the description:
pages 1-58, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____

[X] the claims:
pages 59-61, as originally filed
pages NONE, as amended (together with any statement) under Article 19
pages NONE, filed with the demand
pages NONE, filed with the letter of _____

[X] the drawings:
pages NONE, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____

[X] the sequence listing part of the description:
pages 1-7, as originally filed
pages NONE, filed with the demand
pages NONE, filed with the letter of _____

2. With regard to the **language**, all the elements marked above were available or furnished to this Authority in the language in which the international application was filed, unless otherwise indicated under this item.
These elements were available or furnished to this Authority in the following language _____ which is:

[ ] the language of a translation furnished for the purposes of international search (under Rule 23.1(b)).

[ ] the language of publication of the international application (under Rule 48.3(b)).

[ ] the language of the translation furnished for the purposes of international preliminary examination (under Rules 55.2 and/or 55.3).

3. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international preliminary examination was carried out on the basis of the sequence listing:

[X] contained in the international application in printed form.

[X] filed together with the international application in computer readable form.

[ ] furnished subsequently to this Authority in written form.

[ ] furnished subsequently to this Authority in computer readable form.

[ ] The statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

[ ] The statement that the information recorded in computer readable form is identical to the writen sequence listing has been furnished.

4. [X] The amendments have resulted in the cancellation of:

[X] the description, pages NONE

[X] the claims, Nos. NONE

[X] the drawings, sheets/~~fig~~ NONE

5. [ ] This report has been drawn as if (some of) the amendments had not been made, since they have been considered to go beyond the disclosure as filed, as indicated in the Supplemental Box (Rule 70.2(c)).**

** Replacement sheets which have been furnished to the receiving Office in response to an invitation under Article 14 are referred to in this report as "originally filed" and are not annexed to this report since they do not contain amendments (Rules 70.16 and 70.17).*

***Any replacement sheet containing such amendments must be referred to under item 1 and annexed to this report.*

INTERNATIONAL [redacted]LIMINARY EXAMINATION REPORT

International application No.
[redacted]PCT/US00/05155

III. Non-establishment of opinion with regard to novelty, inventive step and industrial applicability

1. The questions whether the claimed invention appears to be novel, to involve an inventive step (to be non obvious), or to be industrially applicable have not been and will not be examined in respect of:

☐ the entire international application.

☒ claims Nos. 1-23, re: SEQ ID NOS: 2-5, 7-10

because:

☐ the said international application, or the said claim Nos. _ relate to the following subject matter which does not require international preliminary examination *(specify)*.

☐ the description, claims or drawings *(indicate particular elements below)* or said claims Nos. _ are so unclear that no meaningful opinion could be formed *(specify)*.

☐ the claims, or said claims Nos. _ are so inadequately supported by the description that no meaningful opinion could be formed.

☒ no international search report has been established for said claims Nos. (See Attached) .

2. A meaningful international preliminary examination cannot be carried out due to the failure of the nucleotide and/or amino acid sequence listing to comply with the standard provided for in Annex C of the Administrative Instructions:

☐ the written form has not been furnished or does not comply with the standard.

☐ the computer readable form has not been furnished or does not comply with the standard.

INTERNATIONAL PRE[redacted]INARY EXAMINATION REPORT

International application No.
[redacted]/US00/05153

V. Reasoned statement under Article 35(2) with regard to novelty, inventive step or industrial applicability; citations and explanations supporting such statement

1. statement

Novelty (N)	Claims	2, 4-10, 12-23	YES
	Claims	1, 3, 11	NO
Inventive Step (IS)	Claims	2, 4-10, 12-23	YES
	Claims	1, 3, 11	NO
Industrial Applicability (IA)	Claims	1-23	YES
	Claims	NONE	NO

2. citations and explanations (Rule 70.7)

Claim 1 lacks novelty under PCT Article 33(2) as being anticipated by Hillier et al. (XP002147850). The European Search Authority has cited this reference as an "X" reference. However, the description of this prior art in the search report is not clear. It appears that Hillier et al. teach a biologically active or immunogenic fragment of SEQ ID NO:1 and thus anticipates the Claim.

Claim 1 lacks novelty under PCT Article 33(2) as being anticipated by WO 88 01301 A.The European Search Authority has cited this reference as an "X" reference. However, the description of this prior art in the search report is not clear. It appears that WO 88 01301 teaches a biologically active or immunogenic fragment of SEQ ID NO:1 and thus anticipates the Claim.

Claims 3 and 11 lack novelty under PCT Article 33(2) as being anticipated by Hillier et al. (XP002147851).The European Search Authority has cited this reference as an "X" reference. However, the description of this prior art in the search report is not clear. It appears that Hillier et al. teach polynucleotide encoding a biologically active or immunogenic fragment of SEQ ID NO:1 or a polynucleotide comprising at least 60 contiguous nucleitdes of SEQ ID NO:6, or of a polynucleotide having at least 70% identity to SEQ ID NO:6, or complementary nucleotides thereto and thus anticipates the Claim.

Claims 2, 4-10, 12-23 meet the criteria set out in PCT Article 33(2)-(4), because the prior art does not teach or fairly suggest the cells, transgenic organisms, or methods claimed therein, in accordance with the European Search Authority.

On May 23, 2001, a Response to Written Opinion was filed. Applicant believes the present application to be fully in compliance with PCT Articles and Rules, and elects to reserve the right to address these and any other specific objections in the (Continued on Supplemental Sheet.)

Supplemental Box
(To be used when the space in any of the preceding boxes is not sufficient)

Continuation of: Boxes I - VIII

III. NON-ESTABLISHMENT OF REPORT:

No international search report has been established for claim numbers 1-23, re: SEQ ID NOS: 2-5, 7-10 .

V. 2. REASONED STATEMENTS - CITATIONS AND EXPLANATIONS (Continued):
national stage applications to be filed in respective countries.

---------- NEW CITATIONS ----------
NONE

TITLE OF THE INVENTION

DETECTION MARKER FOR COLONIC LESIONS

This invention was made using U.S. Government funds. The U.S. Government has rights in the invention.

Field of the Invention

This invention relates to molecular biological approaches to the diagnosis of premalignant and malignant colonic tissues, especially by nucleic acid hybridization procedures.

Description of the Background Art

Immunological approaches to the diagnosis of premalignant and malignant colonic tissues have been reported by Schlom et al. at the National Institutes of Health of the United States. Thor et al., Nature 311:562 (1984) and Schlom et al., J. Cell. Biochem. Supp. 9A:36 (1985) disclose the detection of specific oncogene products in human colonic tissue. These reports show the use of monoclonal antibody (Mab) to define differential ras gene expression of the p21 protein in benign and malignant colonic diseases. Immunohistochemical analyses of individual cells within tissue sections revealed differences in ras p21 expression in colon carcinomas, compared with normal

colonic epithelium, benign colon tumors, and inflammatory or dysplastic colon lesions. The authors concluded that ras p21 is probably a relatively late event in colon carcinogenesis.

In Thor et al., Lab. Invest. 52:68A (1985); Hand et al., Proc. Nat. Acad. Sci. USA 81:5227 (1984); and Hand et al., Hybridoma 68A (1985), the research group examined a variety of cancer tissues for ras p21 expression using Mab to p21. Ras gene expression was found in 49% of colonic adenosarcoma, 63% of ductal carcinomas, 10% of benign breast tumors, and in 0% of benign colon lesions and normal colons. The majority of human colonic and mammary gland adenocarcinomas exhibited ras gene expression, whereas the majority of abnormal ducts and lobules from fibroadenoma and fibrocystic disease patients were negative. The authors maintained that these findings form the basis of a quantitative RIA for a ras translational product, and provide a means to evaluate ras p21 expression within normal cells, as well as within benign, premalignant, and malignant lesions.

Type C-related human endogenous retroviral sequences have recently been discovered and characterized. They appear to play a role in controlling transcription of linked genes, especially during cell transformation.

Several publications have appeared discussing human endogenous retroviral sequences. For example, Callahan et al., Science 228:1208 (1985), disclose a human recombinant DNA clone (HLM-2) that comprises a mosaic of retroviral-related sequences with the organization and length of known endogenous retroviral genomes. A particular HLM-2 long terminal repeat

(LTR) hybridized with LTR of a type-D retrovirus. The HLM-2 gag and pol genes share extensive homology with a type-A related retrovirus, a type-B retrovirus, and a type-C retrovirus. There were regions in the HLM-2 pol gene that were up to 70% identical to a mammary tumor virus pol gene. A portion of the putative HLM-2 env gene hybridized with the corresponding region of a type-A retroviral genome. Martin et al., PNAS 75:4892 (1982) disclosed the detection and cloning of type-C retroviral sequences in human brain, baboon skin fibroblasts, and rhesus monkey liver.

Rabson et al., Nature 306:604 (1983) disclosed the characterization of a full-length human retroviral clone containing 2 LTR elements 8.4 kb apart, as well as gag, pol, and putative env regions. Hybridization experiments revealed that the human cells (placenta) contained species of poly(A)$^+$ RNA that annealed to segments of the full-length retroviral clone that contains only 4.1 kb of gag-pol sequences, bounded by a tandem array of imperfect repeats 72 to 76 base pairs in length, and lacking LTR's.

Repaske et al., J. Virol. 54:764 (1985) disclosed the complete nucleotide sequence of a full-length (8.8 kb) endogenous C-type human retroviral DNA (clone 4-1) cloned from a human genomic DNA library; colinearity and 40% amino acid homology were found in comparison with Moloney murine leukemia viral DNA.

Rabson et al., J. Virol. 56:176 (1985) disclosed the structure of human endogenous retroviral env RNA transcripts by Northern blot hybridization and cDNA cloning. Poly(A)$^+$ 3.0- and 1.7-kb env RNA's were identified in placenta, colon carcinoma, and breast carcinoma cells.

No accurate nucleic acid - based marker, however, has yet been disclosed to be useful for colonic cancer lesions.

SUMMARY OF THE INVENTION

The present invention is based on the analysis of the pattern of expression of type C-related retroviral sequences in the poly(A)$^+$ RNA extracted from a number of human primary colon cancers, from adjacent colon mucosa and from two colon cancer cell lines (HCT and Caco2), using as probes labeled LTR and envelope sequences (designated H-LTR and H-env). In most of the tumor samples examined, the inventors observed a striking decrease in the amount of a 3.6kb LTR-related transcript, which is very prominent and abundant in normal colon mucosa (NCM); in contrast, either or both of two env-related transcripts of 3.0 and 1.7kb, especially the 1.7kb one, increased in colonic tumors versus NCM.

The present invention thus provides a method for the detection of neoplastic disease of the colon in an RNA containing colonic sample, comprising:

(1) contacting the RNA of the sample with a detectably labeled H-LTR or H-env nucleotide probe; and

(2) detecting the amount of hybridization between the probe and the RNA, relative to the amount in a known, non-cancerous colonic sample.

In a specific preferred embodiment, the invention is directed to a method for analyzing colonic tissues for the transcriptional pattern of type C-related human endogenous retroviral sequences, comprising extracting total RNA from the tissue, recovering poly

$(A)^+$ RNA through oligo-dT cellulose chromatography, size-separating this RNA by denaturing gel electrophoresis, hybridizing with labeled H-LTR or H-*env* human proviral probes, detecting the species hybridized, and quantifying changes in the LTR-specific or *env*-related transcripts.

DESCRIPTION OF THE FIGURES

Figure 1 shows the complete sequence of the *env*-probe.

Figure 2 shows the complete sequence of the H-LTR probe.

DESCRIPTION OF THE PREFERRED EMBODIMENTS

Two nucleic acid probes are used in the method of the invention. The first probe is a sequence identical to or homologous to a sequence from endogenous C-type human retroviral nucleic acid, hereinafter the "envelope" or "*env*" probe. The complete sequence of the envelope type C retroviral gene in question is given in Repaske *et al.*, *J. Virol.* 54:764 (June 1985). The preferred segment is a Bam HI-Hind III fragment thereof, which is shown in Figure 1, or subfragments thereof capable of hybridizing a target nucleic acid.

The second probe is named "H-LTR" and the complete sequence thereof is shown in Figure 2. The H-LTR probe is also derived from the complete sequence of the envelope type C retroviral gene shown in the Repaske *et al.* publication (*supra*). The H-LTR probe is a sequence identical to, part of, or homologous to the sequence shown in Figure 2.

The desired nucleotide probe sequence may include flanking naturally occurring nucleotides as well, with the proviso that these flanking nucleotides may not be

present in such numbers as to alter the hybridization specificity of the DNA or RNA sequence. Typically, the probe sequence will contain at least 18 nucleotides. Thus, further intended within the scope of this invention are any and all polynucleotides containing, as a minimum, 18 members that are part of or homologous to the env or H-LTR probes.

These probes can be either in DNA or in RNA form. They can be obtained by known and published isolation and digestion procedures (supra) or synthesized by standard methods. The probe may be obtained from messenger RNA, from cDNA obtained by reverse transcription of messenger RNA with reverse transcriptase or by cleavage of the genome, conveniently by endonuclease digestion, followed by cloning of the gene or gene fragment in accordance with known techniques. See, for example, Kornberg, DNA Replication, W. H. Freeman & Co., San Francisco, 1980, pp. 670-679. Alternatively, the probe may be synthesized according to the technique described by Merrifield, J. M. Chem. Soc., 85:2149 (1962). After isolation of the DNA fragment, the fragment may be used for preparation of the probe.

The probe can be by itself or may be part of a plasmid. For example, the H-LTR probe is flanked by Taq I sites. It can thus be subcloned into the Taq I site of plasmid pBR322, for example, and cloned in E. coli.

The probe is detectably labelled, the labels of most utility being radioactive atoms, enzymes, chromophores, biotin/avidin, or the like. A more complete discussion of nucleic acid hybridization technology may be found in Huang, E. S. et al., Vol. 6, pp. 457-497 (1977), incorporated by reference herein. Oligonucleotide probe technology is also disclosed by Szostak, J. W. et al., Meth. Enzymol., 68:419-428 (1979), also incorporated by reference herein.

The polynucleotide or oligonucleotide probe may be labelled with an atom or inorganic radical, most commonly using radionuclides, but also perhaps heavy metals. In some situations, it may also be possible to employ an antibody which will bind specifically to the probe hybridized to the target DNA.

Most commonly, a radioactive label is employed, suitable radioactive labels including ^{32}P, ^{3}H, ^{14}C ^{125}I, or the like. Any radioactive label may be employed which provides for an adequate signal and has sufficient half-life. Other labels include ligands, fluorescers, chemiluminescers, enzymes, antibodies, and the like.

In one technique of labelling, E. coli DNA polymerase I may be utilized to add nucleotide residues to the 3'-hydroxy terminus that is created when one strand of a double-stranded DNA molecule is nicked. In addition, the enzyme, by virtue of its 5' to 3' exonucleolytic activity, may remove nucleotides from the 5' side of the nick. The elimination of nucleotides from the 5' side and the sequential addition of nucleotides to the 3' side results in the formation of the nick (nick translation) along the DNA (Kelley et al., J. Biol. Chem., 245: 39 (1970)). By replacing the preexisting nucleotides with highly radioactive nucleotides, it is possible to prepare labelled probe with a specific activity well in excess of 10^8 cpm/ug (Rigby, P. W. J. et al., J. Mol. Biol., 113: 237 (1977)).

Probes maybe labelled to high specific activity using either ^{3}H-thymidine triphosphate or alpha-^{32}P-deoxynucleotide triphosphates by such nick translation (Rigby et al., supra).

In testing a colonic sample for the marker(s), RNA can be isolated from tissue by sectioning on a cryostat and lysing the sections with a detergent such as SDS and a chelating agent such as EDTA, optionally with overnight digestion with proteinase K (50 ug/ml). Protein is removed by phenol and chloroform extractions, and nucleic acids are precipitated with ethanol. RNA is isolated by chromotography on an oligo dT column and then eluted therefrom. Further fractionation can also be carried out.

A number of techniques for molecular hybridization are used for the detection of retroviral RNA sequences in colonic tissues; each has certain advantages and disadvantages. When large amounts of tissue are available, analysis of hybridization kinetics provides the opportunity to accurately quantitate the amount of retroviral RNA present, as well as to distinguish sequences that are closely related but not identical to the probe, and determine the percent homology.

Reactions are run under conditions of hybridization (Tm-25°C) in which the rate of reassociation of the probe is optimal (Wetmur, J. G. et al., J. Mol. Biol., 31: 349-370 (1968)). The kinetics of the reaction are second- order when the sequences in the tissue are identical to those of the probe; however, the reaction exhibits complex kinetics when probe sequences have partial homology to those in the tissue (Sharp, P. A. et al., J. Mol. Biol., 86: 709-726 (1974)).

The concentration of probe to cell RNA is determined by the sensitivity desired. To detect one retroviral transcript per cell would require about 100 pg of probe per ug of total cellular RNA. The nucleic

acids are mixed, denatured, brought to the appropriate salt concentration and temperature, and allowed to hybridize for various periods of time. The rate of reassociation can be determined by quantitating the amount of probe hybridized either by hydroxy apatite chromatography (Britten, R. J. et al., Science, 161: 529-540 (1968)) or S1 nuclease digestion (Sutton, W. D., Biochim. Biophys. Acta, 240: 522-531 (1971)).

A more flexible method of hybridization is the Northern blot technique. This technique offers variability in the stringency of the hybridization reaction, as well as determination of the state of the retroviral sequences in the specimen under analysis. Cell RNA is denatured in situ with alkali, neutralized and transferred to a nitrocellulose membrane.

After washing, the membrane is baked under vacuum and prehybridized in 10X Denhardts solution (0.2% each of Ficoll, bovine serum albumin, polyvinylpyrollidone) in 4X SSC (SSC = 0.15M NaCl, 0.05M sodium citrate) containing 50 ug/ml sonicated and denatured salmon sperm DNA for four hours at 60°C. Stringent hybridization or non-stringent hybridization can be carried out. Membranes are washed extensively in 4X SSC at 52°C, air dried and detected.

A major consideration associated with hybridization analysis of retroviral RNA sequences is the degree of relatedness the probe has with the sequences present in the specimen under study. This is important with the blotting technique, since a moderate degree of sequence homology under nonstringent conditions of hybridization can yield a strong signal even though the probe and sequences in the sample represent different retroviral types.

The particular hybridization technique is not essential to the invention, any technique commonly used in the art being within the scope of the present invention. Typical probe technology is described in United States Patent 4,358,535 to Falkow et al., incorporated by reference herein.

The labelled probes, as described above, provide a general diagnostic method for detection of colonic lesions. The method is reasonably rapid, has a simple protocol, has reagents which can be standardized and provided as commercial kits, and allows for rapid screening of large numbers of samples.

In one method for carrying out the procedure, a clinical isolate containing RNA transcripts is fixed to a support. The affixed nucleic acid is contacted with a labelled polynucleotide having a base sequence complementary or homologous to the coding strand of the retroviral gene.

The hybridization assays of the present invention are particularly well suited for preparation and commercialization in kit form, the kit comprising a carrier means compartmentalized to receive one or more container means (vial, test tube, etc.) in close confinement, each of said container means comprising one of the separate elements to be used in the hybridization assay.

For example, one vial may contain soluble, detectably labelled HLR or env probe, while one or more different vials may contain different, predetermined amounts of env or HLR RNA transcripts. The latter containers may be used to construct a standard curve for interpolating data obtained from the unknown sample.

The presence of colonic lesions is determined by the variation in the appearance and/or quantity of probe-related RNA transcripts in tested tissue. Two LTR specific transcripts and three env-related transcripts are observed.

Table 1 below shows the pattern of transcript distribution in normal colonic mucosa (NCM) versus cancerous colonic tissue.

TABLE I

	NCM	Cancerous Tissue
LTR related transcripts		
3.6 kb	↑↑	↓↓
2.1 kb	-	-
Env - related transcripts		
3.0 kb	-	↑
1.7 kb	-	↑↑
0.6 kb	-	↑

The 1.7 kb env-related transcript clearly increases, and the 3.6 kb LTR-related transcript clearly decreases in cancerous colonic tissue.

Generally a significant increase or decrease, respectively, of these transcripts should be observed for an accurate diagnosis. Preferably a 3-fold or higher for the 1.7 kb transcript, and a 60% or more decrease for the 3.6 kb transcript, respectively should be observed.

Certain examples are now provided for illustration purposes only.

EXAMPLES

Materials and Methods

Cell Cultures, Chemicals and Tissue Specimens.

HCT and Caco2 colon cancer cell lines were cultured in Dulbecco's modified Eagle's medium (DMEM) supplemented with 20% fetal calf serum (GIBCO). Cell cultures were grown in a humidified incubator at 37°C with 5% CO_2 in air and fed three times a week. For DNA and RNA extraction, colon tissue surgical specimens were used, which were not needed for routine pathology and in accordance with the NIH guidelines on human studies.

Probes and Nucleic Acid Hybridizations.

In the initial studies the H-LTR and H-env, cloned in pBR322, were [^{32}P] labeled by nick-translation. In later experiments, the H-LTR segment was subcloned in the pSP64 vector system (Melton, D.A. et al., Nuc. Ac. Res. 12:7035 (1984)) to generate a [^{32}P] labeled riboprobe. Chromosomal DNA was extracted from cell cultures or tissue specimens following a previously described procedure (Gattoni, S., Mol. Cell. Biol. 2:42-51 (1982)), subjected to restriction endonuclease (New England Biolabs), digestion, and to Southern blot hybridization. Total RNA was extracted using the method described by Cathala et al. (DNA 2:329-335 (1983)); poly(A)$^+$ RNA was selected through oligo-dT cellulose chromatography (Aviv, H. et al., PNAS 69:1408-1412 (1972)), size separated by denaturing gel electrophoresis (Thomas, P.A., PNAS 77:5201-5208 (1980)) and blot hybridized (Melton, supra, Wahl, G.M. et al. PNAS 76:3683-3687 (1979)). DNA and RNA blots were subsequently rinsed and exposed for autoradiography using X-ray films (KODAK) at-70°C.

EXAMPLE 1

Northern Blot Analyses

The findings of Rabson, Martin and coworkers (Nature 305:604-607 (1983)), that H-LTR and H-env probes detect specific transcripts in a series of human tissues and cell lines was confirmed. The attention was then focused on normal colon mucosa (NCM), a number of primary colon tumors (T) and two colon cancer cell lines - HCT and Caco2 - from which RNA was extracted and poly(A)$^+$ RNA was selected and size-separated on denaturing agarose gels for Northern blot analysis. In all samples, the H-env probe identified three transcripts of 3.0, 1.7 and 0.6 kb, while the H-LTR probe gave rise to marked differences both in the quality and intensity of hybridization. The H-LTR probe identified a major 3.6 kb species which was very prominent in NCM and T1; in comparison, T3 and T4 showed hardly any hybridization (comparable amounts of RNA were loaded in each lane) and HCT showed much less intense hybridization than the normal colon mucosa. Moreover, the 3.6 kb species did not appear to cross hybridize with H-env, nor with a gag-pol probe.

It was subsequently confirmed that every colon RNA sample analyzed thus far (whether from normal mucosa or tumor), exhibited the three env-specific transcripts, although there was some difference in the intensity of hybridization (tumors seemed to contain consistently more of the 1.7 kb and sometimes more of the 3.0 and 0.6 kb species). In addition, of the four colon cancer cell lines tested (HCT, Caco2, SW480 and Colo320), only the HCT and Caco2 showed the above pat-

tern and have been studied further. In this respect, it should be noted that HCT and Caco2 exhibit a distinct epithelial morphology which has not been observed in stocks of SW480 and Colo 320. The H-env probe, therefore, appears to identify a rather specific pattern of hybridization which has not been detected in a number of human fibroblastic and cancer cell lines; moreover it provides a useful marker for checking RNA integrity. Such a control is important for validating the dramatic decrease of the 3.6 kb LTR transcript in a majority of the colon tumors and in all the colon cancer cell lines examined to date.

A substantially improved resolution of RNA transcripts as well as the sensitivity of the H-LTR probe were also achieved. The LTR insert was subcloned in the pSP6 vector (Melton, supra) and the corresponding riboprobe was used for reprobing filters previously hybridized with a nick-translated H-env probe. An example of such an experiment is one wherein five different poly(A)$^+$ RNAs - two NCM controls, two malignant tumors (T5, T6) and one villoglandular polyp (P1) - were probed with H-env and H-LTR riboprobe. H-env gave the usual pattern (3.0, 1.7 and 0.6 kb transcripts), although the intensity of the signal for the 1.7 and 0.6 kb species was stronger in the tumors. In P1 all three H-env transcripts were clearly increased.

Reprobing the same filters with H-LTR confirmed the previous findings but also provided evidence to suggest that alteration in the transcription of endogenous retroviral sequences may constitute an early event in the process of carcinogenesis. Specifically, a consistent number of differences emerged in both

premalignant and malignant tumors, compared to every sample of normal colon mucosa (NCM) studied thus far. There was a decrease, often very striking, in the amount of the LTR specific 3.6 kb RNA and an increase in the 1.7 kb transcript, sometimes accompanied by a parallel increase in the 3.0 and 0.6 kb species.

In this regard, it is of interest that in the one case of dysplasia in chronic ulcerative colitis (D1) examined thus far, the 1.7 kb transcript was clearly increased (see Table 2 below). (A total colectomy was later performed on this patient and a carcinoma "in situ" was identified in the colon specimen.)

The 3.0 and 1.7 kb RNAs appeared to cross-hybridize with both env and LTR probes. The reason for their being virtually undetectable in NCM using the LTR probe may derive from differential homology between the probe and the LTR portion of the 3.6 kb RNA compared to the 3.0 and 1.7 kb RNAs. Therefore, only when the latter two transcripts were increased above a given threshold did they become detectable. Alternatively, each probe may have identified different, but comigrating transcripts.

The summary of the analyses is shown in Table 2.

TABLE 2
Summary of Northern blot analyses of human colon tissue and colon cancer cell lines

Surgical Specimens	LTR Transcripts 3.6 kb	2.1 kb	LTR-env Transcripts 3.0 kb	1.7 kb	env Transcript 0.6 kb
NCM*	++++	+	±	±§§	+
T1**	++++	+	+	++	++
T2	+	+	-	+	++
T3	±	+	±	+	+
T4	±	+	±	+	+
T5	+	+	±	+	++
T6	+++	+	±	+	++
T7	+	±	+	+	++
T8	++	+	-	+	+
T9	+++	+	±	+	++
T10	±	+	+	+	++
T11	+	+	±	+	+
T12	+	+	+	++	ND
T13	+	+	+	+	ND
T14	++	+	±	+	ND
T15	+	+	+	+	+++
P1***	+	+	+	+	++
D1§	+++	+	-	+	+
Cell lines					
HCT	+	±	++	++	++
CaCo2 CA-I	+	+	-	++	+
CaCo2 CA-II	-	+	±	++	+
CaCo2 CA-III	-	+	±	++	+

* Normal Colon Mucosa: a sample of normal mucosa (NCM) adjacent to each tumor specimen was analyzed and no detectable differences in the transcription pattern of such controls were found.

** T1-14 were colon carcinomas; T15 was a rectal carcinoma.

*** P1 villoglandular polyp

§ D1 dysplasia in chronic ulcerative colitis.

§§ The ± symbol = barely detectable; ND = not done.

The remarkable abundance of the 3.6 kb H-LTR specific transcript and its striking decrease in most of the primary human colon cancers tested suggests that the regulation of the corresponding sequence is altered in tumors in contrast to normal colon mucosa. Colon tumors, on the other hand, appear to express higher levels of the env-related transcripts, especially the 1.7 kb species, including the one case of dysplasia examined thus far. This observation and the finding of a "cancer-like" pattern in a non-metastatic, non-malignant tumor (P1) indicates that type C-related endogenous retroviral sequences provide early detection markers of colonic lesions progressing to neoplasia. In particular, the increase of the 1.7 kb RNA and the often dramatic decrease of the 3.6 kb species represent the best indices of an altered transcription pattern corresponding to an altered phenotype.

COMPARATIVE EXAMPLE 1

Examination of Retroviral Sequences in Genomic DNA

Southern blot hybridization of the genomic pattern of type C-related human endogenous retroviral sequences was analyzed in a number of DNA samples derived either from human colon carcinoma cell lines or from human colon surgical specimens.

H-LTR was used as a probe to compare the pattern of integration of homologous genomic sequences in seven different chromosomal DNA samples extracted from a human fibroblastic cell line (HF), four colon cancer cell lines (Caco2, SW480, Colo320 and HCT), a specimen of normal colon mucosa (NCM) and a colon tumor (T1).

The Southern blot hybridization of these PstI digested chromosomal DNAs indicated that the H-LTR probe identified multiple homologous sequences in the human genome, as was previously shown with a gag-env probe (Steele, P.E., Science 225:943-847 (1984)).

Although the complexity of the hybridization pattern did not allow detection of minor variations, there appeared to be no obvious difference among the DNAs examined.

EXAMPLE 2

Studies on Human Colon Cancer Cell Lines

In parallel, the HCT and Caco2 cell lines were also characterized, since they share several markers and morphological features with intestinal epithelia. In addition, confluent Caco2 cells have been shown to express a large increase in the levels of intestinal alkaline phosphatase as well as markers of enterocytic differentiation. In this respect Caco2 cells provide an inducible system, susceptible to in vitro manipulation.

Therefore, Caco2 cells were cultured and extracted for RNA at different times after seeding - namely, in exponential growth (CA-I) at subsconfluence (CA-II) and at confluence (CA-III). The corresponding poly(A)$^+$ RNAs were subjected to Northern blot hybridization with the H-LTR riboprobe and compared with the normal mucosa control (NCM), the HCT cell line, and two primary tumors (T7 and T8).

The 3.6 kb transcript was again very prominent in NCM and was decreased in both primary tumors and HCT cells. However, the Caco2 cells appeared to produce the 3.6 kb transcript only when growing exponentially.

The transcriptional pattern of these sequences in HCT and Caco2 cells is remarkable similar to the pattern observed in some primary tumors (see Table 2). T7 exhibits both env-related transcripts (3.0 and 1.7 kb) and is therefore comparable to the HCT cells in which these RNAs are rather prominent; T8 instead, shows only the smaller transcript as is the case in Caco2 cells, although in subconfluent and confluent cultures very small amounts of the 3.0 kb transcript are detectable.

DISCUSSION OF EXAMPLES

The first consistent finding was the increase in the LTR-env 1.7 kb transcript in all primary tumors and in the two non-malignant lesions tested (i.e. polyp and dysplasia).

The second relevant finding is the decrease, often very striking, of the 3.6 kb LTR-related transcript. The "in vitro" studies with the Caco2 cell line showed that the expression of such a transcript can be modulated, suggesting that the corresponding gene product may be involved in the process of differentiation of colon mucosal cells.

The variability of the transcription pattern in tumors and tumor cell lines as compared to the remarkable consistent pattern in 15 normal colon mucosal specimens is also compatible with the concept of tumor heterogeneity, which encompasses morphological as well as biochemical features of cancer, especially primary ones.

The present results indicate that an altered pattern of expression of retroviral transcripts provides markers for the detection of the neoplastic disease at its initial stages.

WHAT IS CLAIMED IS:

1. A method for the detection of neoplastic disease of the colon in an RNA containing colonic sample, which comprises:

(1) contacting the RNA of said sample with a detectably labeled H-LTR or H-env nucleotide probe; and

(2) detecting the amount of hybridization between the probe and the RNA, relative to the amount in a known, non-cancerous, colonic sample.

2. The method of claim 1 wherein said probe is H-env.

3. The method of claim 1 wherein said probe is H-LTR.

4. The method of claim 2 wherein said H-env probe has at least a portion, or is homologous to a portion, of the sequence of Figure 1.

5. The method of claim 3 wherein said H-LTR probe has at least a portion, or is homologous to a portion, of the sequence of Figure 2.

6. The method of any of claims 4 or 5 wherein said portion is at least 18 nucleotides long.

7. The method of claim 2 which comprises detecting a significant increase in a 1.7 Kb retroviral RNA transcript present in said sample relative to said non-cancerous sample.

8. The method of claim 3 which comprises detecting a significant decrease in a 3.6 Kb retroviral RNA transcript present in said sample relative to said non-cancerous sample.

9. The method of claim 1 which comprises, prior to said step (1), purifying RNA from said sample.

10. The method of claim 9 wherein said RNA is purified by oligo-dT chromatography.

11. The method of claim 1 wherein said probe is labeled with a radioactive atom, an enzyme, or a biotin or avidin label.

FIG. 1

```
BAMHI                                                   6120
GGATCCACCACAGCCGTGTAAAACCTGCAGTGCCT
rpIleHisHisSerArgValLysProAlaValPre
                                                         ENV
GAAACCTGGGAGGCAAGACCAAGCCCAGAAAACCCCTGCAGAGTGACCCCGAAGAAGACAACAAGCCCTGCTCCAGTCACACCCGGAAGCTGACTGGTCCACGCACGGCCGAAGCATGCAG
GlyThrTrpGluAlaArgProSerProGluAsnProCysArgValThrProLysLysThrThrSerProAlaProValThrProGlySer###                       MetGln
                                                                                                                    6360
AAGCTCATCATGGGATTCATTTTTCTTAAATTTTGGACTTATACAGTAAGGGCTTCAACTGATCTTACTCAAACTGGGGACTGTTCCCAGTGTATTCATCAGGTCACCHAGGTAGGACAG
LysLeuIleMetGlyPheIlePheLeuLysPheTrpThrTyrThrValArgAlaSerThrAspLeuThrGlnThrGlyAspCysSerGlnCysIleHisGlnValThrGluValGlyGln
                                                                                                                    6480
CAAATTAAAACAATGTTTCTGTTCTATAGTTATTATAAATGTATAGGAACATTAAAAGAAACTTGTTTGTATAATGCTACTCAGTACAATGTATGTAGCCCAGGAAATGACCGACCTGAT
GlnIleLysThrMetPheLeuPheTyrSerTyrTyrLysCysIleGlyThrLeuLysGluThrCysLeuTyrAsnAleThrGlnTyrAsnValCysSerProGlyAsnAspArgProAsp
                                                                                                                    6600
GTGTGTTATAACCCATCTGAGCCTCCTGCAACCACCATTTTTGAAATAAGAATAAGAACTGGCCTTTTCCTAGGTGATACAAGTAAAATAATAACTAGAACAGAAGAAAAAGAAATCCCC
ValCysTyrAsnProSerGluProProAlaThrThrIlePheGluIleArgIleArgThrGlyLeuPheLeuGlyAspThrSerLysIleIleThrArgThrGluGluLysGluIlePro
                                                                                                                    6720
AAACAAATAACTTTAAGATTTGATGCTTGTGCAGCCATTAATAGTAAAAAGCTAGGAATAGGATGTGATTCTCTTAACTGGGAAAGGAGCTACAGAATAAAAAATAAATATGTTTGTCAT
LysGlnIleThrLeuArgPheAspAlaCysAlaAlaIleAsnSerLysLysLeuGlyIleGlyCysAspSerLeuAsnTrpGluArgSerTyrArgIleLysAsnLysTyrValCysHis
                                                                                                                    6840
GAGTCAGGGGTTTGTGAAAATTGTGCCTATTGGCCATGTGTTATTTGGGCTACTTGGAAAAAGAACAAAAAGGACCCGGTTTATCTTCAGAAGGGGGAAGCCAACCCCTCCTGTGCTGCT
GluSerGlyValCysGluAsnCysAlaTyrTrpProCysValIleTrpAlaThrTrpLysLysAsnLysLysAspProValTyrLeuGlnLysGlyGluAlaAsnProSerCysAlaAla
                                                                                                                    6960
GGTCACTGTAACCCACTAGAACTAATAATTACCAATCCCCTAGATCCCCATTGGAAAAAGGGAGAACGTGTAACCCTGGGGATTGATGGGACAGGGTTAAACCCCCAAGTTGCCATTTTA
GlyHisCysAsnProLeuGluLeuIleIleThrAsnProLeuAspProHisTrpLysLysGlyGluArgValThrLeuGlyIleAspGlyThrGlyLeuAsnProGlnValAlaIleLeu
                                                                                                                    7080
ATTAGAGGGGAGGTCCACAAGTGCTCTCCCAAACCAGTATTTCAAACCTTTTATAAGGAGCTGAATCTGCCAGCACCAGAATTTCCAAAAAAGACAAAAAATTTGTTTCTCCAATTAGCA
IleArgGlyGluValHisLysCysSerProLysProValPheGlnThrPheTyrLysGluLeuAsnLeuProAlaProGluPheProLysLysThrLysAsnLeuPheLeuGlnLeuAla
                                                                                                                    7200
GAAAATGTAGCTCATTCCCTTAATGTTACTTCTTGTTATGTATGCGGGGGAACCACTATCGGAGACCGATGGCCTTGGGAAGCCCGAGAGTTGGTGCCTACTGATCCAGCTCCTGATATA
GluAsnValAlaHisSerLeuAsnValThrSerCysTyrValCysGlyGlyThrThrIleGlyAspArgTrpProTrpGluAlaArgGluLeuValProThrAspProAlaProAspIle
                                                                                                              HINDIII 7320
ATTCCAGTTCAGAAAACCCAAGCTAGCAACTTCTGGGTCCTAAAAACCTCAATTATTGGACAATACTGTATAGCTAGAGAAGGGAAAGACTTTATCATCCCTGTAGGAAAGCTT
IleProValGlnLysThrGlnAlaSerAsnPheTrpValLeuLysThrSerIleIleGlyGlnTyrCysIleAlaArgGluGlyLysAspPheIleIleProValGlyLysLeu
```

1/2

AGCTAGATGACCTTGGCACCACCACCTGGCCCTGGTGGCTAAAATATAATATTATTAACCCCTGACCAAAACTGTTGGTGTTATCTGTAAATTCCAGATATTGTATGAGA

360

AGTACTGTAAAACTTTTTATTCTGTTAGCTGATGTAGGTAGCCCCCAGTCATGTTTCTCACGCTTACTTGACCTATTATGACTTTTTCATGTAGACCCCTTAGAGTTGTAAGCCCTTAA

480

AGGGCTAGGAATTTCTTTTTTGGGGAGCTCGGCTCTTAAGATACGAGTCTGCCAATGCTCCCGGCCAAATAAAAAACCTCTTCCTTCTTTAATCTGGTGTCTGAGGAGTTTTGTCTGTG

600

CTCGTCCTGCTACATTTCTTGGTTCCCTGGCCAGGAAGCAAGGTAATTGAAGGACAGTCGAGGCAGCCCCTTAGGTGGCTTAGGCCTGCCCTGTGGAGCATCCCTGCAGGGGACTCTGG

CAGCT

FIG. 2

INTERNATIONAL SEARCH REPORT

International Application No PCT/US 87/01889

I. CLASSIFICATION OF SUBJECT MATTER (if several classification symbols apply, indicate all) [6]

According to International Patent Classification (IPC) or to both National Classification and IPC

IPC[4]: C 12 Q 1/68; C 12 N 15/30

II. FIELDS SEARCHED

Minimum Documentation Searched [7]

Classification System	Classification Symbols
IPC[4]	C 12 Q 1/00; C 12 N 15/00; G 01 N 33/00

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched [8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT [9]

Category *	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No. [13]
Y	EP, A, 0097341 (IMREG INC.) 4 January 1984 see page 3, line 17 - page 4, line 13; page 53, line 18 - page 54, line 29; page 55, lines 3-21 --	1-9,11
Y	EP, A, 0117727 (PRESIDENT & FELLOWS OF HARVARD COLLEGE) 5 September 1984 see page 2, lines 9-16; page 5, lines 3-18 --	1-9,11
Y	EP, A, 0186522 (SANKYO CO. LTD.) 2 July 1986 see page 2, lines 18-23; page 3, line 18 - page 4, line 34; page 14, lines 6-20; page 17, line 2 - page 19, line 24 --	1-9,11
X,P	Proceedings National Academy of Sciences USA, vol. 83, 16 August 1986 Medical Sciences S. Gattoni-Celli et al.: "Expression of type C-related endogenous retro-	./.

* Special categories of cited documents: [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
27th November 1987	11 JAN 1988
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	M. VAN MOL

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category *	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
	viral sequences in human colon tumors and colon cancer cell lines", pages 6127 - 6131, see page 6127, column 1, lines 1-27; page 6127, column 2, line 25 - page 6128, column 1, line 2	1-11
	--	
X	Chemical Abstracts, vol. 105, 29 June 1986 (Columbus, Ohio, US) J.A. Moshier et al.: "mRNA from human colon tumor and mucosa related to the pol gene of an endogenous A-type retrovirus ", see page 178, abstract no. 219846a, & Biochem. Biophys. Res. Commun. 1986, 139(3), 1071-7	1-3
	--	
X	Chemical Abstracts, vol. 100, 9 April 1984 (Columbus, Ohio, US) L.H. Augenlicht et al.: "Elevated expression of an endogenous retroviral long terminal repeat in a mouse colon tumor", see page 127, abstract no. 115704h, & J. Biol. Chem. 1984, 259 (3), 1842-7	1-3
	------	

(12) INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(19) World Intellectual Property Organization
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(63) Related by continuation (CON) or continuation-in-part (CIP) to earlier application:
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Filed on 1 March 1999 (01.03.1999)

(71) Applicant *(for all designated States except US)*: **INCYTE PHARMACEUTICALS, INC.** [US/US]; 3160 Porter Drive, Palo Alto, CA 94304 (US).

(72) Inventors; and

(75) Inventors/Applicants *(for US only)*: **LAL, Preeti** [IN/US]; 2382 Lass Drive, Santa Clara, CA 95054 (US). **YUE, Henry** [US/US]; 826 Lois Avenue, Sunnyvale, CA 94087 (US). **HILLMAN, Jennifer, L.** [US/US]; 230 Monroe Drive #12, Mountain View, CA 94040 (US). **LU, Dyung, Aina, M.** [US/US]; 55 Park Belmont Place, San Jose, CA 95136 (US). **BAUGHN, Mariah, R.** [US/US]; 14244 Santiago Road, San Leandro, CA 94577 (US). **TANG, Y., Tom** [CN/US]; 4230 Ranwick Court, San Jose, CA 95118 (US). **AZIMZAI, Yalda** [US/US]; 2045 Rock Springs Drive, Hayward, CA 94545 (US).

(74) Agents: **HAMLET-COX, Diana** et al.; Incyte Pharmaceuticals, Inc., 3160 Porter Drive, Palo Alto, CA 94304 (US).

(81) Designated States *(national)*: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW.

(84) Designated States *(regional)*: ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published:
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29 March 2001

For two-letter codes and other abbreviations, refer to the "Guidance Notes on Codes and Abbreviations" appearing at the beginning of each regular issue of the PCT Gazette.

WO 00/52161 A3

(54) Title: LEUKOCYTE- AND BLOOD-ASSOCIATED PROTEINS

(57) Abstract: The invention provides human leukocyte- and blood-associated proteins (LBAP) and polynucleotides which identify and encode LBAP. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with expression of LBAP.

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INTERNATIONAL APPLICATION PUBLISHED UNDER THE PATENT COOPERATION TREATY (PCT)

(51) International Patent Classification 7 :
C12N 15/12, C07K 14/705, C12N 5/10, A01K 67/027, C12P 21/02, C07K 16/28, C12Q 1/68, A61K 38/17, G01N 33/53

A2

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60/122,080 1 March 1999 (01.03.99) US

(63) Related by Continuation (CON) or Continuation-in-Part (CIP) to Earlier Application
US 60/122,080 (CIP)
Filed on 1 March 1999 (01.03.99)

(71) Applicant *(for all designated States except US)*: INCYTE PHARMACEUTICALS, INC. [US/US]; 3160 Porter Drive, Palo Alto, CA 94304 (US).

(72) Inventors; and
(75) Inventors/Applicants *(for US only)*: LAL, Preeti [IN/US]; 2382 Lass Drive, Santa Clara, CA 95054 (US). YUE, Henry [US/US]; 826 Lois Avenue, Sunnyvale, CA 94087 (US). HILLMAN, Jennifer, L. [US/US]; 230 Monroe Drive #12, Mountain View, CA 94040 (US). LU, Dyung, Aina, M. [US/US]; 55 Park Belmont Place, San Jose, CA 95136 (US). BAUGHN, Mariah, R. [US/US]; 14244 Santiago Road, San Leandro, CA 94577 (US). TANG, Y., Tom [CN/US]; 4230 Ranwick Court, San Jose, CA 95118 (US). AZIMZAI, Yalda [US/US]; 2045 Rock Springs Drive, Hayward, CA 94545 (US).

(74) Agents: HAMLET–COX, Diana et al.; Incyte Pharmaceuticals, Inc., 3160 Porter Drive, Palo Alto, CA 94304 (US).

(81) Designated States: AE, AL, AM, AT, AU, AZ, BA, BB, BG, BR, BY, CA, CH, CN, CU, CZ, DE, DK, EE, ES, FI, GB, GD, GE, GH, GM, HR, HU, ID, IL, IN, IS, JP, KE, KG, KP, KR, KZ, LC, LK, LR, LS, LT, LU, LV, MD, MG, MK, MN, MW, MX, NO, NZ, PL, PT, RO, RU, SD, SE, SG, SI, SK, SL, TJ, TM, TR, TT, UA, UG, US, UZ, VN, YU, ZA, ZW, ARIPO patent (GH, GM, KE, LS, MW, SD, SL, SZ, TZ, UG, ZW), Eurasian patent (AM, AZ, BY, KG, KZ, MD, RU, TJ, TM), European patent (AT, BE, CH, CY, DE, DK, ES, FI, FR, GB, GR, IE, IT, LU, MC, NL, PT, SE), OAPI patent (BF, BJ, CF, CG, CI, CM, GA, GN, GW, ML, MR, NE, SN, TD, TG).

Published
Without international search report and to be republished upon receipt of that report.

(54) Title: LEUKOCYTE– AND BLOOD–ASSOCIATED PROTEINS

(57) Abstract

The invention provides human leukocyte– and blood–associated proteins (LBAP) and polynucleotides which identify and encode LBAP. The invention also provides expression vectors, host cells, antibodies, agonists, and antagonists. The invention also provides methods for diagnosing, treating, or preventing disorders associated with expression of LBAP.

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INTERNATIONAL SEARCH REPORT

Inter ɔnal Application No
PCT/US 00/05153

A. CLASSIFICATION OF SUBJECT MATTER
IPC 7 C12N15/12 C07K14/705 C12N5/10 A01K67/027 C12P21/02
C07K16/28 C12Q1/68 A61K38/17 G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HILLIER ET AL.: "The washU-Merck EST Project" EMBL SEQUENCE DATABASE, 18 February 1996 (1996-02-18), XP002147850 HEIDELBERG DE Ac N51814 the whole document ---	1
X	HILLIER ET AL.: "WashU-Merck EST project 1997" EMBL SEQUENCE DATABASE, 13 June 1997 (1997-06-13), XP002147851 HEIDELBERG DE Ac AA460341 the whole document --- -/--	3,11

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 September 2000	05. 1. 01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	CEDER O.

Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Inter ional Application No
PCT/US 00/05153

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 88 01301 A (GEN HOSPITAL CORP) 25 February 1988 (1988-02-25) abstract; figures	1
A	US 5 861 272 A (LI YI ET AL) 19 January 1999 (1999-01-19) column 1, line 5 - line 17 column 2, line 20 -column 3, line 30	1-17,20, 23
A	EP 0 346 710 A (MOLECULAR DIAGNOSTICS INC) 20 December 1989 (1989-12-20) abstract	1,3,6,8, 9

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

International application No.
PCT/US 00/05153

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [X] Claims Nos.: 18, 19, 21, 22
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-23 all partly

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-23 all partly

Isolated polypeptide and polynucleotide sequences and their uses where the sequences are Seq Id Nos 1 and 6.

2. Claims: 1-23 all partly

Isolated polypeptide and polynucleotide sequences and their uses where the sequences are Seq Id Nos 2 and 7.

3. Claims: 1-23 all partly

Isolated polypeptide and polynucleotide sequences and their uses where the sequences are Seq Id Nos 3 and 8.

4. Claims: 1-23 all partly

Isolated polypeptide and polynucleotide sequences and their uses where the sequences are Seq Id Nos 4 and 9.

5. Claims: 1-23 all partly

Isolated polypeptide and polynucleotide sequences and their uses where the sequences are Seq Id Nos 5 and 10.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 16, 19 and 22 are directed to methods of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.1

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

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Continuation of Box I.2

Claims Nos.: 18, 19, 21, 22

Claims 18,9,21,22 refers to agonists/antagonists of the polpeptide of claim 1 without giving a true technical characterization. Moreover, no such compounds are specifically defined in the description. It is only indicated that they "may include proteins" (such as antibodies)", nucleic acids, carbohydrates, small molecules, or any other compound or compostion wich modulates the activity of LBAP either by directly interacting with LBAP or by acting on components of the biological pathway in which LBAP parcipitates." (page 6 lines 19-21; page 7 lines 20-23). In consequence the scope of said claims is ambiguous and vague, and their subject-matter is not sufficiently disclosed and supported (Art. 5 and 6 PCT). No search can be carried out for such claims whose wording is, in fact, a mere recitation of the results to be achieved.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

Information on patent family members

Inte onal Application No
PCT/US 00/05153

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 8801301 A	25-02-1988	EP 0318502 A	07-06-1989
		JP 2500323 T	08-02-1990
US 5861272 A	19-01-1999	NONE	
EP 0346710 A	20-12-1989	US 5122599 A	16-06-1992
		AT 97162 T	15-11-1993
		AU 628348 B	17-09-1992
		AU 3644389 A	21-12-1989
		DE 68910590 D	16-12-1993
		DE 68910590 T	17-03-1994
		DK 296189 A	17-12-1989
		ES 2059621 T	16-11-1994
		FI 892910 A	17-12-1989
		IE 62161 B	28-12-1994
		IL 90594 A	20-11-1997
		JP 2107190 A	19-04-1990
		NO 892220 A	18-12-1989
		NZ 229514 A	28-05-1991
		US 6013772 A	11-01-2000
		US 6022958 A	08-02-2000
		ZA 8904553 A	28-03-1990

PATENT COOPERATION TREATY

PCT

INTERNATIONAL SEARCH REPORT

(PCT Article 18 and Rules 43 and 44)

Applicant's or agent's file reference	FOR FURTHER ACTION	see Notification of Transmittal of International Search Report (Form PCT/ISA/220) as well as, where applicable, item 5 below.
PF-0673 PCT		
International application No.	International filing date *(day/month/year)*	(Earliest) Priority Date *(day/month/year)*
PCT/US 00/05153	29/02/2000	01/03/1999
Applicant		
INCYTE PHARMACEUTICALS, INC.		

This International Search Report has been prepared by this International Searching Authority and is transmitted to the applicant according to Article 18. A copy is being transmitted to the International Bureau.

This International Search Report consists of a total of 6 sheets.

☐ It is also accompanied by a copy of each prior art document cited in this report.

1. **Basis of the report**

   a. With regard to the **language,** the international search was carried out on the basis of the international application in the language in which it was filed, unless otherwise indicated under this item.

   ☐ the international search was carried out on the basis of a translation of the international application furnished to this Authority (Rule 23.1(b)).

   b. With regard to any **nucleotide and/or amino acid sequence** disclosed in the international application, the international search was carried out on the basis of the sequence listing :

   ☒ contained in the international application in written form.

   ☐ filed together with the international application in computer readable form.

   ☐ furnished subsequently to this Authority in written form.

   ☒ furnished subsequently to this Authority in computer readble form.

   ☒ the statement that the subsequently furnished written sequence listing does not go beyond the disclosure in the international application as filed has been furnished.

   ☒ the statement that the information recorded in computer readable form is identical to the written sequence listing has been furnished

2. ☒ **Certain claims were found unsearchable** (See Box I).

3. ☒ **Unity of invention is lacking** (see Box II).

4. With regard to the **title,**

   ☒ the text is approved as submitted by the applicant.

   ☐ the text has been established by this Authority to read as follows:

5. With regard to the **abstract,**

   ☒ the text is approved as submitted by the applicant.

   ☐ the text has been established, according to Rule 38.2(b), by this Authority as it appears in Box III. The applicant may, within one month from the date of mailing of this international search report, submit comments to this Authority.

6. The figure of the **drawings** to be published with the abstract is Figure No. _____

   ☐ as suggested by the applicant.

   ☐ because the applicant failed to suggest a figure.

   ☐ because this figure better characterizes the invention.

   ☐ None of the figures.

INTERNATIONAL SEARCH REPORT

International Application No
PCT/US 00/05153

A. CLASSIFICATION OF SUBJECT MATTER

IPC 7 C12N15/12 C07K14/705 C12N5/10 A01K67/027 C12P21/02
C07K16/28 C12Q1/68 A61K38/17 G01N33/53

According to International Patent Classification (IPC) or to both national classification and IPC

B. FIELDS SEARCHED

Minimum documentation searched (classification system followed by classification symbols)
IPC 7 C07K

Documentation searched other than minimum documentation to the extent that such documents are included in the fields searched

Electronic data base consulted during the international search (name of data base and, where practical, search terms used)

C. DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	HILLIER ET AL.: "The washU-Merck EST Project" EMBL SEQUENCE DATABASE, 18 February 1996 (1996-02-18), XP002147850 HEIDELBERG DE Ac N51814 the whole document ---	1
X	HILLIER ET AL.: "WashU-Merck EST project 1997" EMBL SEQUENCE DATABASE, 13 June 1997 (1997-06-13), XP002147851 HEIDELBERG DE Ac AA460341 the whole document --- -/--	3,11

[X] Further documents are listed in the continuation of box C.

[X] Patent family members are listed in annex.

° Special categories of cited documents :

"A" document defining the general state of the art which is not considered to be of particular relevance

"E" earlier document but published on or after the international filing date

"L" document which may throw doubts on priority claim(s) or which is cited to establish the publication date of another citation or other special reason (as specified)

"O" document referring to an oral disclosure, use, exhibition or other means

"P" document published prior to the international filing date but later than the priority date claimed

"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step when the document is taken alone

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

Date of the actual completion of the international search	Date of mailing of the international search report
19 September 2000	05. 1. 01

Name and mailing address of the ISA	Authorized officer
European Patent Office, P.B. 5818 Patentlaan 2 NL - 2280 HV Rijswijk Tel. (+31-70) 340-2040, Tx. 31 651 epo nl, Fax: (+31-70) 340-3016	CEDER O.

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Form PCT/ISA/210 (second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

[redacted]

International Application No
[redacted]T/US 00/05153

C.(Continuation) DOCUMENTS CONSIDERED TO BE RELEVANT

Category °	Citation of document, with indication, where appropriate, of the relevant passages	Relevant to claim No.
X	WO 88 01301 A (GEN HOSPITAL CORP) 25 February 1988 (1988-02-25) abstract; figures ---	1
A	US 5 861 272 A (LI YI ET AL) 19 January 1999 (1999-01-19) column 1, line 5 - line 17 column 2, line 20 -column 3, line 30 ---	1-17,20, 23
A	EP 0 346 710 A (MOLECULAR DIAGNOSTICS INC) 20 December 1989 (1989-12-20) abstract -----	1,3,6,8, 9

1

Form PCT/ISA/210 (continuation of second sheet) (July 1992)

INTERNATIONAL SEARCH REPORT

Information on patent family members

International Application No
PCT/US 00/05153

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
WO 8801301 A	25-02-1988	EP 0318502 A	07-06-1989
		JP 2500323 T	08-02-1990
US 5861272 A	19-01-1999	NONE	
EP 0346710 A	20-12-1989	US 5122599 A	16-06-1992
		AT 97162 T	15-11-1993
		AU 628348 B	17-09-1992
		AU 3644389 A	21-12-1989
		DE 68910590 D	16-12-1993
		DE 68910590 T	17-03-1994
		DK 296189 A	17-12-1989
		ES 2059621 T	16-11-1994
		FI 892910 A	17-12-1989
		IE 62161 B	28-12-1994
		IL 90594 A	20-11-1997
		JP 2107190 A	19-04-1990
		NO 892220 A	18-12-1989
		NZ 229514 A	28-05-1991
		US 6013772 A	11-01-2000
		US 6022958 A	08-02-2000
		ZA 8904553 A	28-03-1990

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

1. Claims: 1-23 all partly

Isolated polypeptide and polynucleotide sequences and their uses where the sequences are Seq Id Nos 1 and 6.

2. Claims: 1-23 all partly

Isolated polypeptide and polynucleotide sequences and their uses where the sequences are Seq Id Nos 2 and 7.

3. Claims: 1-23 all partly

Isolated polypeptide and polynucleotide sequences and their uses where the sequences are Seq Id Nos 3 and 8.

4. Claims: 1-23 all partly

Isolated polypeptide and polynucleotide sequences and their uses where the sequences are Seq Id Nos 4 and 9.

5. Claims: 1-23 all partly

Isolated polypeptide and polynucleotide sequences and their uses where the sequences are Seq Id Nos 5 and 10.

FURTHER INFORMATION CONTINUED FROM PCT/ISA/ 210

Continuation of Box I.1

Although claims 16, 19 and 22 are directed to methods of treatment of the human/animal body, the search has been carried out and based on the alleged effects of the compound/composition.

Continuation of Box I.1

Rule 39.1(iv) PCT - Method for treatment of the human or animal body by therapy

---

Continuation of Box I.2

Claims Nos.: 18, 19, 21, 22

Claims 18,9,21,22 refers to agonists/antagonists of the polpeptide of claim 1 without giving a true technical characterization. Moreover, no such compounds are specifically defined in the description. It is only indicated that they "may include proteins" (such as antibodies)", nucleic acids, carbohydrates, small molecules, or any other compound or compostion wich modulates the activity of LBAP either by directly interacting with LBAP or by acting on components of the biological pathway in which LBAP parcipitates." (page 6 lines 19-21; page 7 lines 20-23). In consequence the scope of said claims is ambiguous and vague, and their subject-matter is not sufficiently disclosed and supported (Art. 5 and 6 PCT). No search can be carried out for such claims whose wording is, in fact, a mere recitation of the results to be achieved.

The applicant's attention is drawn to the fact that claims, or parts of claims, relating to inventions in respect of which no international search report has been established need not be the subject of an international preliminary examination (Rule 66.1(e) PCT). The applicant is advised that the EPO policy when acting as an International Preliminary Examining Authority is normally not to carry out a preliminary examination on matter which has not been searched. This is the case irrespective of whether or not the claims are amended following receipt of the search report or during any Chapter II procedure.

INTERNATIONAL SEARCH REPORT

national application No.
PCT/US 00/05153

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This International Search Report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

see FURTHER INFORMATION sheet PCT/ISA/210

2. [X] Claims Nos.: 18, 19, 21, 22
because they relate to parts of the International Application that do not comply with the prescribed requirements to such an extent that no meaningful International Search can be carried out, specifically:

see FURTHER INFORMATION sheet PCT/ISA/210

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this International Search Report covers all searchable claims.

2. [ ] As all searchable claims could be searched without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this International Search Report covers only those claims for which fees were paid, specifically claims Nos.:

4. [X] No required additional search fees were timely paid by the applicant. Consequently, this International Search Report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

1-23 all partly

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO. US 8701889 SA 18219

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report. The members are as contained in the European Patent Office EDP file on 09/12/87
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information.

Patent document cited in search report	Publication date	Patent family member(s)	Publication date
EP-A- 0097341	04-01-84	WO-A- 8400037	05-01-84
		AU-A- 1820883	16-01-84
		US-A- 4490472	25-12-84
		CA-A- 1211062	09-09-86
EP-A- 0117727	05-09-84	JP-A- 59224566	17-12-84
		US-A- 4542096	17-09-85
		CA-A- 1214092	18-11-86
EP-A- 0186522	02-07-86	JP-A- 61152287	10-07-86